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Expression of recombinant S100A8 subunit and evaluation of Ca effect on its tertiary structure

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Abstract

Background: S100A8 as a subunit of calprotectin heterodimer plays a role in inflammatory processes and cancer.

Objective: The aim of this study was to express recombinant S100A8 and to evaluate Ca effect on its tertiary structure.

Methods: This experimental study was performed in Qazvin University of medical sciences, 2013. Recombinant S100A8 subunit was expressed in pET15b, E. coli BL21 (DE3) system as a his-tagged protein. The protein purification process was accomplished under both native and denaturing conditions using Ni-NTA column and different concentrations of imidazole. The expression and homogeneity of recombinant protein was analyzed using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The tertiary structure of S100A8 was studied in the absence and presence of Ca using fluorescence spectrometry.

Findings: The highest expression of S100A8 with apparent molecular weight of 8 kDa was found in denaturing conditions. In the purification process, the most purified S100A8 was seen with a gradient of 150 and 200 mM imidazole. The maximum fluorescence emission of S100A8 was observed at 330 nm in the presence and absence of calcium. Emission intensity was decreased in the presence of different concentrations of calcium.

Conclusion: Changes in tertiary structure of S100A8 subunit in the presence of calcium may affect the protein function and may contribute to understand the role of this protein in cancer and inflammatory processes.

Keyword: Fluorescence Spectrometry, Calcium, Polyacrylamide Gel Electrophoresis, Plasmids, Proteins

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